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MOLECULAR CLONING AND CHARACTERIZATION OF HUMAN *XRCC2* DNA REPAIR GENE. N. Liu, J.E. Lamerdin, J.D. Tucker, L.S. Narayana¶, M.J. Siciliano¶, A.V. Carrano, Z.-Q. Zhou#, C.A. Walter#, and L.H. Thompson, Lawrence Livermore National Lab., Livermore, CA 94550, ¶Univ. of Texas M.D. Anderson Cancer Center, Houston, TX 77030, #Univ. of Texas Health Science Center at San Antonio, San Antonio, TX 78284

The hamster V79 cell mutant irs1 is hypersensitive to a broad variety of DNA damage, especially DNA cross-links caused by mitomycin C (MMC) and cisplatin, and has elevated chromosomal aberrations (~30%). The human gene that corrects irs1 was named *XRCC2*, based on studies with somatic cell hybrids (1). To isolate the gene, an EBV-derived cDNA expression library (kindly supplied by Dr. R. Legerski), was transfected into irs1 cells. Two transformants that survived at 30 nM MMC and hygromycin B were further characterized as having ~10-fold more resistance to MMC than irs1 cells and cross-resistance to cisplatin and ethyl methanesulfonate. An episomal plasmid with a cDNA insert of ~3 kb was recovered from the Hirt extract of one transformant. The cDNA insert was mapped to chromosome 7q36 by Southern blotting of a hybrid clone panel, which agrees with the localization of the gene using somatic cell hybrids (1). The genomic sequence of *XRCC2* was obtained by screening a P1 library with *XRCC2* cDNA probe. The *XRCC2* cDNA and genomic DNA transformants showed partial correction in terms of cell survival for MMC. With ionizing radiation, the genomic transformant showed wild-type sensitivity while the cDNA transformants surprisingly showed no correction. Chromosomal aberrations showed intermediate correction in both plasmid and genomic transformants in unirradiated cultures as well as after 100 cGy of ^{137}Cs γ-rays. The open reading frame (ORF) in *XRCC2* cDNA consists of 840 bp, encoding 280 amino acids. The *XRCC2* genomic DNA was sequenced almost to completion, showing at least 3 exons, which are separated by two relatively long introns (10-11 kb). The predicted protein sequence shows weak similarity with *S. cerevisiae* RAD51 and contains consensus ATP binding domains. Northern hybridization gave a single transcript of 1.8 kb in baboon tissue, and the level of *XRCC2* gene expression was ~100-fold higher in testis than in the other tissues. The XRCC2 protein has been overexpressed in *E. coli* and purified to near homogeneity. Polyclonal antibodies are being made against XRCC2, and biochemical activities, such as ATPase will be examined. We suggest that XRCC2 may play a role in a homologous recombinational pathway that efficiently repairs DNA interstrand cross-links. (Work was done under the auspices of the U.S. DOE by LLNL under contract No. W-7405-ENG-48).

1. Jones, N. J., Zhao, Y., Siciliano, M. J., and Thompson, L. H. (1995) Assignment of the *XRCC2* human DNA repair gene to chromosome 7q36 by complementation analysis. Genomics 26, 619-622.